Certificate of Analysis

pHTC HaloTag® CMV-neo Vector:

Part No.	Size
G771A	20µg

Part# 9PIG771

Revised 1/18

Description: The pHTC HaloTag® CMV-neo Vector[a–c] is configured to append the HaloTag® tag to the carboxy-terminus of the fused protein. The vector provides constitutive high-level protein expression in mammalian cells using the human cytomegalovirus (CMV) immediate early enhancer/promoter. The vector contains a multiple cloning region for convenient cloning and can be used for both transient and stable gene expression. The stable expression is mediated by co-expression of the neomycin phosphotransferase gene, which confers resistance to the antibiotic G-418 (Cat.# V7983). Alternatively, the HaloTag® coding region and the accompanying linker can be removed (either using PCR or restriction enzyme digestion) and transferred into other commercially available vectors.

Note: The insert must contain an in-frame ATG codon for translation initiation.

The pHTC HaloTag® CMV-neo Vector contains the following features:

- A **CMV immediate-early enhancer/promoter** for constitutive expression in mammalian cells.
- A **T7 RNA polymerase promoter** for in vitro HaloTag® fusion protein expression in cell-free systems (e.g., TnT® lysate reaction).
- A **multiple cloning region** containing unique restriction sites to facilitate gene insertion into the vector.
- The **C-terminal HaloTag® region**, which rapidly forms covalent bonds with HaloTag® ligands and surfaces, enabling labeling and immobilization of expressed proteins.
- A **HaloTag® linker,** a stretch of amino acids that allows efficient flexibility of the HaloTag® tag when fused to the protein of interest.
- A **TEV protease site** for cleavage of the expressed protein from HaloTag® coding region using TEV protease.
- An **ampicillin-resistance gene** for selection of plasmid in bacteria.

Concentration: 1µg/µl.

GenBank® Accession Number: JF920305.

Storage Buffer: The pHTC HaloTag® CMV-neo Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 7.4).

Storage Conditions: See Product Information Label for storage recommendations and expiration date. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability.

Usage Notes:

1. For stable expression, the transfected cells must be selected with the antibiotic G-418. Following transfection, seed the cells at low density, and apply the G-418 antibiotic to the medium at a concentration 100µg/ml–1mg/ml. For effective selection, the cells should be subconfluent; nongrowing cells are resistant to the effects of G-418. The concentration of G-418 required to select and maintain drug resistance depends on the cell type and growth rate. In general, mammalian cells require a concentration of 400–600µg/ml of G-418 for selection and 200–400µg/ml of G-418 for maintenance of stable transfectants. Change the growth medium every 3 days until drug-resistant clones appear (2–5 weeks, depending on the cell type). For cells not expressing neomycin phosphotransferase, cell death should occur 3–9 days after adding G-418.
2. When removing the HaloTag® gene to insert into other vectors, it is critical to also include the HaloTag® linker and the TEV protease recognition sequence to ensure best function of the HaloTag® coding region.

Quality Control Assays

Contaminant Assays

Contaminating Nucleic Acids: RNA, single-stranded DNA and chromosomal DNA are not evident in an overload sample of this vector as determined by agarose gel electrophoresis.

Nuclease Assay: To demonstrate the absence of endonucleases and exonucleases, vector DNA is incubated in standard digest buffers at 37°C for 16 hours followed by agarose gel electrophoresis. The specification is <10% conversion to nicked or linear DNA.

Physical Purity: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$.

Functional Assays

Identity Assay: The vector has been sequenced completely and has 100% identity with the published sequence available at: **www.promega.com/products/vectors**

Restriction Enzyme Digests: Vector DNA is analyzed for the presence of certain restriction enzyme sites by incubation with a variety of restriction enzymes at the specified digestion temperature for 1 hour. Samples are examined by agarose gel electrophoresis, comparing cut and uncut vector DNA with marker DNA.

Signed by:

R. Wheeler, Quality Assurance

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Usage Information

pHTC HaloTag® CMV-neo Vector Features and Circle Map

The following features are present in the pHTC HaloTag® CMV-neo Vector based on nucleotide sequence.

CMV intermediate early enhancer/promoter	1–742
Chimeric intron	857–989
T7 RNA polymerase promoter (–17 to +3)	1033–1052
Multiple cloning region	1052–1129
HaloTag® linker	1124–1168
TEV protease recognition sequence	1139–1159
HaloTag® coding region	1169–2059
SV40 late polyadenylation signal	2177–2398
SV40 enhancer and early promoter	2497–2915
SV40 minimum origin of replication	2813–2878
Neomycin phophotransferase coding region	2960–3754
Synthetic polyadenylation signal	3818–3866
β-lactamase (Amp^r) coding region	4127–4987
*Col*E1-derived plasmid origin of replication	5142–5178

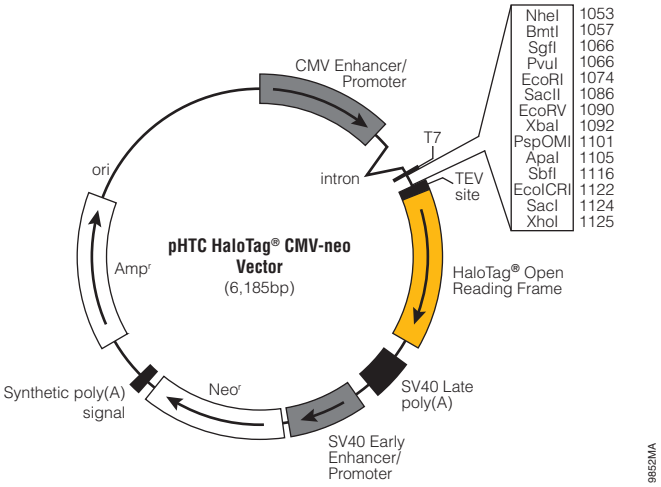

Figure 1. pHTC HaloTag® CMV-neo Vector circle map and sequence reference points.

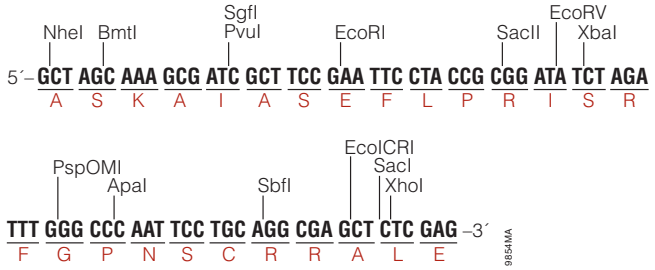

Figure 2. pHTC HaloTag® CMV-neo Vector multiple cloning region sequence and unique restriction sites. The amino acid sequence corresponds to the correct reading frame for the HaloTag® coding region.

Related Products

Product	Size	Cat.#
JM109 Competent Cells, >10^8cfu/µg	5 × 200µl	L2001
JM109 Competent Cells, >10^7cfu/µg	5 × 200µl	L1001
HB101 Competent Cells, >10^8cfu/µg	5 × 200µl	L2011
HaloTag® Mammalian Protein Detection and Purification System	1 each	G6795
HaloTag® Mammalian Pull-Down and Labeling System	24 reactions	G6500
HaloCHIP™ System	20 reactions	G9410

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